ISSN 1063-0740, Russian Journal of Marine Biology, 2019, Vol. 45, No. 4, pp. 292–301. © Pleiades Publishing, Ltd., 2019.
Russian Text © The Author(s), 2019, published in Biologiya Morya.

The Sperm Ultrastructure and Some Reproductive Characteristics of the Chemosymbiotic Bivalve *Calyptogena pacifica* Dall, 1891 (Vesicomyidae: Pliocardiinae)

A. L. Drozdov[a, d], *, E. M. Krylova[b], A. A. Kudryavtsev[c, e], S. V. Galkin[b], and S. A. Tyurin[a, d]

[a]*National Scientific Center of Marine Biology, Far East Branch, Russian Academy of Sciences, Vladivostok, 690041 Russia*
[b]*Shirshov Institute of Oceanology, Russian Academy of Sciences, Moscow, 117997 Russia*
[c]*Zoological Institute, Russian Academy of Sciences, St. Petersburg, 199034 Russia*
[d]*Far Eastern Federal University, Vladivostok, 690950 Russia*
[e]*St. Petersburg State University, St. Petersburg, 199034 Russia*
**e-mail: anatoliyld@mail.ru*

Received August 7, 2018; revised February 7, 2019; accepted February 7, 2019

Abstract—Pliocardiines (Bivalvia: Vesicomyidae: Pliocardiinae) are a chemosymbiotrophic group of bivalve mollusks that are obligate for reducing environments. These mollusks house endosymbiotic thioautotrophic bacteria in their gills, which provide nutrition for the host. The ultrastructure of spermatozoa and the state of the gonads in the pliocardiine bivalve *Calyptogena pacifica* in June 2016 were studied. Material was collected in the Bering Sea on the slopes of the Piip's Volcano at a depth of 466 m. The condition of the gonads indicated a pre-spawning state. Active processes of spermatogenesis and oogenesis were noted in the gonads. The mature spermatozoon has an elongated bullet-shaped head with an average length of 4 ± 0.2 μm from the tip of the acrosome to the base of the mid-piece. The mid-piece was formed by a complex of four spherical mitochondria with a diameter of approximately 0.7 μm. An electron dense material of a lipid nature was observed in the distal region of the mid-piece of the sperm. *C. pacifica* mature eggs are approximately 200 μm in diameter. The results are discussed in the context of the available data on the morphology of pliocardiine gametes.

Keywords: reproduction, vesicomyids, chemosymbiosis-based communities, gonads, gametogenesis, spermatozoa, Piip's Volcano, northwestern Pacific

DOI: 10.1134/S1063074019040047

INTRODUCTION

Reducing marine environments occurring in areas of hydrothermal vents and cold seeps contrast with the background in their physico-chemical parameters. Such biotopes and associated chemosynthesis-based communities, although they have a pronounced island-type pattern of distribution, are widespread in the World Ocean. Some chemosymbiotrophic species that are obligate for reducing conditions are characterized by extensive transoceanic distribution patterns; however, their habitats are separated by vast areas that are unsuitable for the life of adults. In these cases, reproductive biology is especially important for understanding the mechanisms of dispersal of obligate chemosymbiotrophic animals and formation of the fauna of the fragmented reducing environments.

Pliocardiines (Bivalvia: Vesicomyidae: Pliocardiinae) are one of the conspicuous symbiotrophic groups. To date, the subfamily includes more than 110 species [31]. They are distributed in the World Ocean from the shelf to oceanic trenches and inhabit all types of reducing environments [36]. There are 20 genera of Pliocardiinae [38]; however, the generic assignment of many species is ambiguous, and despite using morphological and molecular characters, the taxonomy of the subfamily has not been finalized.

It is known that the characters of the structure of spermatozoa are among the most phylogenetically informative for bivalve mollusks [5, 14]. Nevertheless, information on pliocardiine gamete morphology in the literature is scarce. According to published data [23, 43], Pliocardiinae exhibit a sperm type adapted for external fertilization, the so-called primitive [21], or classical [5] sperm. This type of flagellated sperm is characterized by a barrel-shaped, bullet-shaped, or cylindrical head, consisting of a nucleus with an acrosome, and a mid-piece with two mutually perpendicular centrioles surrounded by four mitochondria; a tail flagellum arises from the distal centriole. Morphological features of sperm that could be characteristic of individual genera of Pliocardiinae are not known.

One of the largest genera in the subfamily is the genus *Calyptogena* Dall, 1891 with ten species [35]. The type species of the genus, *C. pacifica* Dall, 1891, is widely distributed in the Pacific Ocean: it is found in the eastern Pacific along the North American coast from Alaska to California, as well as in the northwestern Pacific in the Bering Sea on the slope of the Piip's Volcano [35] and on the slope of the Koryak Highland [3, 34]. *C. pacifica* usually occurs at depths of 500–900 m, although its records are known from a depth of 361 m (northern California) down to 2423 m (Juan de Fuca Ridge, northeastern Pacific) [10, 35].

The literature provides some data on the structure of the reproductive system and spermatozoa of *C. pacifica* from the Monterey Bay, California [41], as well as another species of the genus *Calyptogena, C. gallardoi* from off the coast of Chile [43]. In this paper, we examined the sperm ultrastructure and the state of the gonads in June 2016 in individuals of *C. pacifica* from the slopes of the Piip's Volcano. Newly obtained data and previously published information on the sperm and egg cell structure, as well as on the synchrony and seasonality of reproductive processes in Pliocardiinae are discussed in the comparative aspect. Knowledge on the sperm morphology of different representatives of Pliocardiinae could help reproductive biology may improve understanding the mechanisms of maintaining the extensive disjunct ranges of pliocardiines.

MATERIALS AND METHODS

For the study of the reproductive system, material was obtained from two mature specimens of *Calyptogena pacifica* collected in the Bering Sea in the area of the submarine Piip's Volcano during the 75th cruise of the R/V *Akademik Lavrentyev.* Bivalves were collected with a manipulator of the ROV Comanche 18 (station LV-75-15, 55°382′ N, 167°261′ E, 466 m, June 16, 2016, IO RAS). For verification of taxonomic identification, 14 ethanol-fixed specimens of *C. pacifica* collected at the same station were used.

Immediately after collection, mollusks were placed in a container with outboard sea water. Pieces of the gonads were sampled within several hours after specimens had been collected. Prior to fixation, gonad fragments and the contents of the mantle cavity were examined with a Leica DM IL inverted light microscope. For ultrastructural study of spermatozoa, pieces of the testis were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer supplemented with sodium chloride to achieve the tonicity of seawater. The material was postfixed in 1% OsO_4 in cacodylate buffer, dehydrated in alcohols according to the standard procedure, and embedded in an epon-araldite mixture. Ultrathin sections were cut on an Ultracut ultratome (Reichert). Sections were sequentially stained with uranyl acetate and lead citrate, then observed in a Carl Zeiss Libra 120 electron microscope.

Collection Area

The submarine Piip's Volcano is located in the Commander Basin of the Bering Sea, 75 km from Bering Island and 335 km from the Kamchatka Peninsula. The volcano has a southern (447 m) and a northern (360 m) peaks, which are 2 miles apart from each other and are separated by a saddle with a maximum depth of 650 m [9]. The bivalves *C. pacifica* were found at depths of 466–489 m at the southern peak along the cracks in the hydrothermal deposits covered with a layer of greenish-gray sediment. Low-temperature methane-enriched fluids are thought to emanate from the cracks [9].

RESULTS

The Morphology of Adult Mollusks

The shells were strongly corroded (Fig. 1). In some specimens, the subumbonal parts of the hinge edge and areas of the adductor scars had holes penetrating the shell wall. The hinge teeth were developed normally. On the right valve, the hinge consists of a ventral cardinal tooth, a subumbonal cardinal tooth with an anterior and a broad posterior rami, as well as a nymphal ridge. The left valve has an anterior subumbonal cardinal tooth with an anterior and a posterior rami, as well as a posterior cardinal tooth, which is longer than half of the nymph. The shells of all specimens have a deep escutcheon and a clearly visible pallial line without a sinus.

All investigated bivalves were dioecious with external sexual dimorphism. Shells of males are usually smaller and more elongated with a sloping postero-dorsal margin, while in females the shells are broadly oval, with a less sloping posterior dorsal margin (Fig. 1).

Structure of the Reproductive System

Mature eggs are located in the ovaries in the central part of tubules, and relatively numerous growing oocytes are located adjacent to the tubule walls along the periphery. Most of the observed oocytes have not yet completed growth and are not ready for spawning. Mature eggs reach 200 μm in diameter (Fig. 2) and are surrounded by two membranes: a yolk membrane 2 μm thick and an outer membrane of 7 μm. The egg cytoplasm is filled with numerous yolk granules and lipid droplets.

The testes of the studied mollusks are organized as a system of tubules and acini. The walls of the testes are lined with a germinal epithelium and auxiliary cells. The germinal epithelium includes gametogenic cells at different stages of development: spermatogonia, spermatocytes, and spermatids. Spherical spermatocytes with a granular cytoplasm and a nucleus occupy most of the acini. The spermatocytes are approximately 6 μm in diameter. Mature spermatozoa are localized in the lumen of the acini. In unpreserved

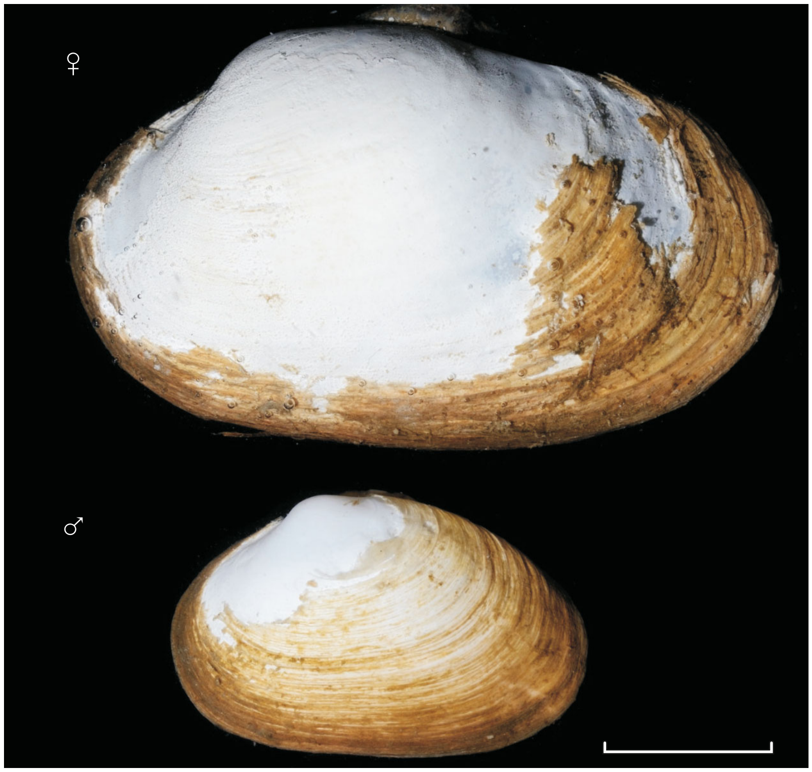

Fig. 1. The shells of adult *Calyptogena pacifica*, left view. Scale bar: 1 cm.

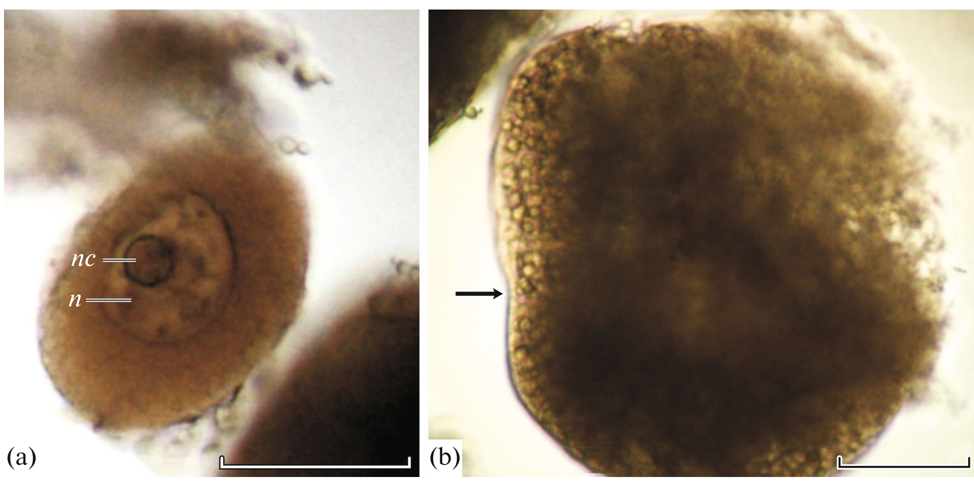

Fig. 2. Oocytes of *Calyptogena pacifica*: (a) growing oocyte; (b) mature egg cell (the integrity is broken). *n*, nucleus; *nc*, nucleolus, arrow indicates an egg cell membrane. Scale bar: 50 μm.

material, spermatocytes and mature spermatozoa were easily washed out from the testes (Fig. 3).

Mature spermatozoa have an elongated bullet-shaped head 4.3 ± 0.2 μm long from the tip of the acrosome to the base of the mid-piece (Figs. 3–5). The mid-piece of the sperm is formed by a complex of four spherical mitochondria with a diameter of approximately 0.7 μm each, interconnected via inter-mitochondrial junctions (Figs. 4f, 4g). Mitochondria are surrounded by two mutually perpendicular centrioles: proximal and distal. The proximal centriole lies in an invagination of the distal part of the nucleus, in the centriolar fossa. The distal centriole is surrounded by a pericentriolar complex that holds it in the distal part of the sperm. The tail flagellum approximately 25 μm long arises from the distal centriole. A ring of homogeneous electron-dense material, presumably of a lipid nature, is located distal to the mitochondria in the mid-piece.

The elongated-conical acrosome approximately 1.5 μm long is located in the apical part of the head. The acrosome consists of two parts: an elongated dome-shaped acrosomal vesicle filled with electron-dense homogeneous material and a granular subacrosomal material adjacent to the nucleus. The nucleus is truncated-conical, with a slightly convex apical part.

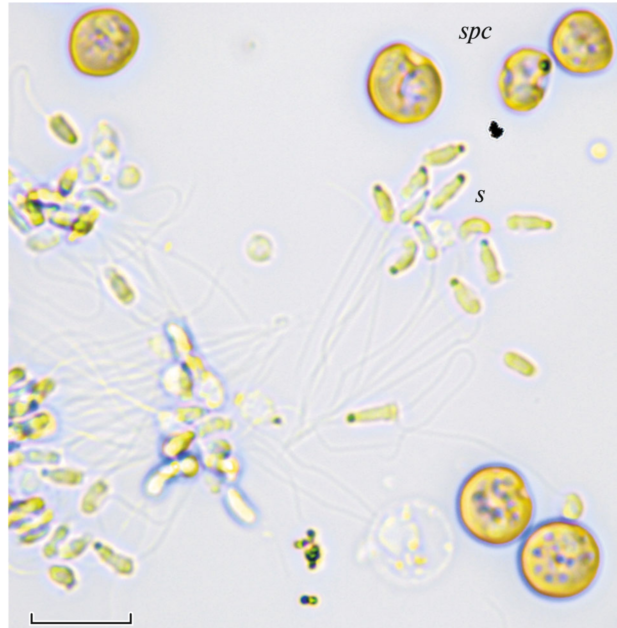

Fig. 3. Mature spermatozoa and spermatogenic cells of *Calyptogena pacifica* washed out of the gonads. Living material. *s*, sperm cells; *spc*, spermatogenic cells. Scale bar: 10 μm.

DISCUSSION

Morphology of Adult Mollusks

Analysis of the morphological features of mollusks studied here and comparison with previously collected specimens of *Calyptogena pacifica* from the slopes of the Piip's Volcano [35], as well as unpublished molecular data (S. Sharina, unpublished data) indicate the correctness of the identification of the specimens used in the study as *C. pacifica.*

C. pacifica that inhabits the slope of Piip's Volcano exhibits external sexual dimorphism, which was previously described in this species from other locations [16, 35]. Since sexual dimorphism, as evident in the same variations of the shell shape, was shown for *C. gallardoi* [43] and some more species of the genus *Calyptogena* [35], it can be supposed that the dimorphism is characteristic of the genus as a whole.

Reproductive Cycle

Judging by the morphology of the gonads, mollusks from the slope of the Piip's Volcano were in the pre-spawning state in mid-June 2016. In the presence of mature gametes, an active gametogenesis was observed in both females and males. This is consistent with the data obtained for *C. pacifica* occurring in a hydrocarbon seep area in Monterey Bay (California) at a depth of 600 m [41] (Table 1). During 3 months, from August to October 1994, gametes at different stages of development, including egg cells and spermatozoa ready for spawning, were present in the gonads; the quantitative indices of development of reproductive tissues varied slightly. The simultaneous presence of gametes at different stages of development, including mature ones, was also noted for *Phreagena soyoae* (cited as *Calyptogena kilmeri,* [41]), an undescribed species of Pliocardiinae from the Blake Ridge [29], for *"Calyptogena" magnifica* [13], and *C. gallardoi* [43]. The asynchronous growth of oocytes indicates an annual reproductive cycle with multiple spawning or with continuous spawning, which is not suggestive of a lack of seasonality in reproduction.

Studies over longer periods of time indicate possible seasonal differences in the intensity of spawning in Pliocardiinae. As an example, from August to November 1994 and in March 1995, *Ph. soyoae* (cited as *Calyptogena kilmeri* [41]) occurring in a hydrocarbon seep area in Monterey Bay at a depth of 900 m showed an increase in the average diameter of oocytes [41]. Variations in the proportion of reproductive and somatic tissues in the female gonads of *Ph. soyoae* from June 1994 to March 1995 also indicate seasonality in reproductive activity with peaks in November and March; however, a certain number of mature oocytes were present in the gonads during the entire period of study [41]. Despite the seasonal differences, spawning is probably possible throughout the year. Thus, 11 spawning events over 1.5 years caused by a slight increase in temperature were reported for *Ph. soyoae* from Sagami Bay (depth 1175 m) [23]. Males were the first to spawn, followed by females, as a rule, within the next 10 min [23]. No seasonal timing of spawning was observed.

Size of Oocytes

According to our data, mature oocytes of *C. pacifica* from the Piip's Volcano slope are up to 200 μm, which falls into the size range of oocytes of *C. pacifica* from Monterey Bay [41]. However, oocyte sizes are similar in different species of Pliocardiinae (Table 1). The oocytes of pliocardiines are among the largest for bivalves [12]. According to traditional views, such dimensions correspond to lecithotrophic or direct

Fig. 4. Spermatozoa of *Calyptogena pacifica*, transmission electron micrographs. (a) Longitudinal section of sperm cell; (b) longitudinal section of acrosome; (c) transverse section of acrosome of spermatid; (d) transverse section of distal part of acrosome of sperm; (e, f, g) transverse sections of sperm mid-piece: (e) an electron-dense lipid droplet is visible, (f) four mitochondria surround the distal centriole with a flagellum arising from it, (g) mitochondria surround mutually perpendicular proximal and distal centrioles. Designations: *ac*, acrosomal complex; *av*, acrosomal vesicle; *dc*, distal centriole; *f*, flagellum; *imc*, intermitochondrial connection; *lm*, lipid material; *m*, mitochondrion; *n*, nucleus; *pc*, proximal centriole; *pm*, subacrosomal material. Scale bars: (a) 1 μm; (b), (c), (d), (e), (f), (g) 0.5 μm.

types of development [6, 42], which implies a brief planktonic larval stage. In this aspect, the assumption of Beninger and Le Penneck [12] that the nutrient reserves in large oocytes of the symbiotrophic bivalve *Acharax alinae* (family Solemyidae) could contribute to increasing the lifespan of the lecithotrophic larva is very interesting. Data have accumulated that indicate that the species distribution ranges of pliocardiines are rather extensive, despite very special ecological requirements and the spatial discontinuity of environments suitable for their habitation [10, 17, 37]. The presence of a larva with an extended developmental period would explain the genetic homogeneity, which is often observed in spatially disjunct subpopulations

Table 1. Characterization of the reproductive system in pliocardiine bivalves (Vesicomyidae: Pliocardiinae)

Species	Size of mature oocytes, μm	Maximum length of head with mid-piece; maximum length of flagellum, μm	Shape of nucleus of spermatozoon; number of mitochondria	Shape and length of acrosome	Oocytes at different stages of growth in a specimen: present (+) or absent (–); time of observation (if indicated)	Seasonality of spawning	Sampling area
"*Calyptogena*" *magnifica*	309–482 [15] 100–200 [40]	3; 8.5 [12]	Barrel-shaped; 4 (KS)	–	+; February 1979 [15]	–	Galapagos Rift, 2450 m [?12, 15]; 9° N, ETP, 2515 m [40]; Gulf of California, Alarcon Rise, 2293 m (KS)
Abyssogena phaseoliformis	180 [40]	4.8 [40]	Barrel-shaped; 4 [19]	–	+ [19]	–	Japan Trench, 5130–5695 m [19, 40]
"*Archivesica*" *laubieri*	–	–	–	–	+ [19]	–	Tenryu Canyon, 3800–4020 m [19]
Phreagena soyoae		4.5 [23]	Barrel-shaped; 4 [23]	Dome-shaped; 0.8 μm [23]	+ [18]	No; 11 Spawning events over 1.5 years [23]	Sagami Bay, 1160 m [18], 1175 m [23]
Phreagena soyoae (как *Calyptogena kilmeri*)	180–237 [41]	–	–	–	+; June 1994 – March1995 [41]	More intensive spawning in fall and spring than in summer [41]	Monterey Bay, 900 m [41]
Phreagena okutanii	210–250 [30]	–	–	–	+ [30]	–	Sagami Bay, 856–1055 m [30]
Pliocardia sp.	200 [29]	–	–	–	+ [29]	–	Western Atlantic, Blake Ridge, 2155 m [29]
Calyptogena gallardoi	273.78±23.12 [43]	3.7 ± 0.2; 26.5 ± 2.5 [43]	Truncated-conical; 4 [43]	Elongated-conical [43]	+ [43]	–	Eastern Pacific, Conception Bay, 760–800 m [43]
Calyptogena pacifica	180–220 [41] 200 (++)	3.5; 12 [12] 4.3 ± 0.2; 25 (++)	Truncated-conical; 4 (++)	Elongated-conical; 1.5 μm (++)	+; August–October 1994 [41]; + (++)	? [41]	? [12]; Monterey Bay, 600 m [41]; Bering Sea, Piip's Volcano, 466 m (++)

Note: The figure in parentheses is a literature source; ++, present study; KS, Krylova and Sahling, *in press*.

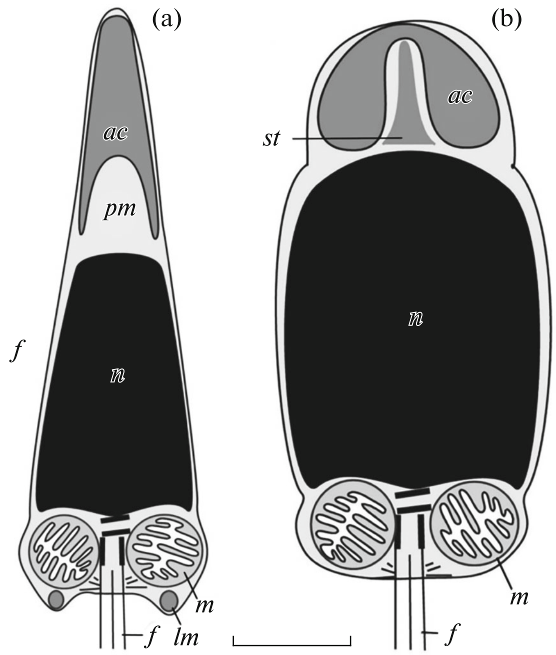

Fig. 5. The scheme of the structure of the spermatozoa of *Calyptogena pacifica* (a) and *Phreagena soyoae* (b) [by 23]. *st*, axial rod; other designations, as in Fig. 4. Scale bar: 1 μm.

of pliocardiines [39]. It is possible that, in the case of Pliocardiinae, as well as *A. alinae,* the classical concept of lecithotrophic larva with a short planktonic stage needs to be revised.

Structure of Spermatozoa

The general type of structure of the spermatozoa in *C. pacifica* does not differ from that of most bivalves, particularly those belonging to the order Venerida. These are typical flagellated spermatozoa, as characteristic of animals with external fertilization [5, 6, 20, 22, 26, 45].

The images obtained in our study confirm the presence of intermitochondrial connections (IMCs) in the sperm of *C. pacifica*. IMCs have been noted in the spermatozoa of bivalves such as *Modiolus modiolus* [32] and *Glycymeris yessoensis* [8]; they can be seen in images of sperm in *Mytilus edulis, Atactodea striata, Modiolus rumphii*, and *Eucrassatella cumingii* [14, see Fig. 24B–E]. In addition to spermatozoa, IMCs have been found in cells of actively functioning animal tissues, such as muscle [1, 2] and nerve tissues [7]. For the spermatozoa of Vesicomyidae, IMCs were noted for the first time. The IMC ultrastructure is universal among different groups; IMCs have the same parameters and morphological features: the gap between mitochondria is 15–20 nm, the size of particles forming the transverse connections between mitochondria is 6–8.5 nm, and the distance between particles is up to 13–18 nm [7]. It was suggested that the IMCs are necessary for the formation of the energy system of the cell, the mitochondriome, which allows rapid distribution of the energy reserves in the volume of the cell [46].

In specimens we examined, a ring of electron-dense material located distal to the mitochondria was found for the first time for vesicomyid spermatozoa. This structure is presumably of a lipid nature. Lipid material in the mid-piece of the sperm has been noted in different groups of animals. In particular, two lipid droplets were found in the sperm mid-piece of the sand dollar *Scaphechinus griseus* (Clypeasteroida) [4]. Apparently, lipids provide an additional source of energy for sperm, ensuring the prolongation of the active phase.

Comparative Analysis of Pliocardiine Sperm

Despite sharing a common structural plan, the spermatozoa of different groups of bivalves differ in particular ultrastructural features [6, 27, 28]. Specific features of sperm structure are among the most informative morphological characters in the systematics of bivalve mollusks [14]. The system and phylogeny of Pliocardiinae have been intensively developed using morphological and molecular methods [17, 31, 38]; however, the relationships between many genera have not been resolved thus far. The use of features of sperm structure can be productive for solving the taxonomic problems of the group. We compiled all of the available information on the structure of the pliocardiine sperm, including the data that were first obtained in the present study for *C. pacifica* from the Bering Sea (Table 1). The sizes of the head and flagellum previously reported for *C. pacifica* by Beninger and Le Pennec [12], significantly differ from our measurements (Table 1). In particular, according to the data of the authors, the flagellum of *C. pacifica* sperm is two times shorter than in the specimens we studied. We believe that the discrepancy in size may be due not to variability of the sperm within the same species, but rather to the incorrect identification of the species. At the time of writing [12], the taxonomic uncertainty in the family Vesicomyidae was very high at both the species and generic levels (see discussion in [35]). Molecular studies have demonstrated a large number of cryptic species within the family [25, 44]. Moreover, the above authors [12] did not indicate the locality where their material was collected; therefore, even indirect evidence is not suggestive of the reliability of species identification.

Information on the size, number of mitochondria, and the nucleus shape of spermatozoa has been published for five species from four genera of Pliocardiinae: C. *gallardoi, C. pacifica, Ph. soyoae, "Calyptogena" magnifica*, and *Abyssogena phaseoliformis*; while on the shape and length of the acrosome, only for *C. gallardoi, C. pacifica,* and *Ph. soyoae*. The maximum length of the sperm head varies from 3 μm (*Calyptogena magnifica*) to 4.8 μm (*A. phaseoliformis*); the maximum length of the flagellum, from 8.5 μm ("*Calyptogena*" *magnifica*) to 26.5 ± 2.5 μm (*C. gallardoi*). All studied species have four mitochondria in the midpiece of the spermatozoon. The nucleus is truncated conical in shape in both species of the genus *Calyptogena*, while it is barrel-shaped in representatives of other genera. The second character separating both species of the genus *Calyptogena* is the elongated-conical shape of the acrosome. Spermatozoa of *Ph. soyoae* differ from the sperm of *C. gallardoi* and *C. pacifica* in having a dome-shaped acrosome and a rod in the subacrosomal material [23, Fig. 5].

Species of the genera *Calyptogena* and *Phreagena* often form dense beds in close proximity to each other [11, 24]. It was shown that the spawning in *Ph. soyoae* can be induced by an increase in temperature of more than 0.1°C [23]. Obviously, a change in temperature is not a species-specific stimulus and causes spawning in different species of bivalves living in the immediate vicinity. In the case of mass spawning of different species with external fertilization, significant differences in the structure of spermatozoa may be of particular importance, reducing the risk of fertilization of the eggs of another species, formation of hybrids, and the loss of valuable reproductive material.

The differences in the structure of the spermatozoa in mollusks of the genera *Calyptogena* and *Phreagena* support the earlier conclusions on the distant affinity of the genera, based on the morphological characteristics of the shells and soft body [33, 35], as well as based on molecular markers [31]. The data obtained in our work for the first time indicate that the ultrastructural features of spermatozoa can be used to diagnose genera within the subfamily Pliocardiinae.

ACKNOWLEDGMENTS

The authors are grateful to the expedition leader V.V. Ivin, master V.B. Ptushkin, the crew of the R/V *Akademik Lavrentyev*, as well as to V.A. Denisov and the crew of technicians of the ROV Comanche 18 for their expert help. We are deeply grateful to Yoshihiro Fujiwara (JAMSTEC, Japan), who sent us TEM images of *Phreagena soyoae* spermatozoa.

FUNDING

This research was performed within the framework of the state assignment project (no. 0149-2019-0009). E.M. Krylova (comparative analysis of reproductive characteristics of pliocardiines) and S.V. Galkin (collection of material) were supported by the Russian Foundation for Basic Research (project no. 18-05-60228).

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. This article does not contain any studies involving animals performed by any of the authors.

REFERENCES

1. Bakeeva, L.E. and Chentsov, Yu.S., Mitochondrial reticulum: structure and some functional properties, *Itogi Nauki Tekh., Ser.: Obshch. Probl. Biol.*, 1989, vol. 9.
2. Bakeeva, L.E., Chentsov, Yu.S., and Skulachev, V.P., Intermitochondrial contacts of cardiomyocytes, *Tsitologiya*, 1982, vol. 245, pp. 161–166.
3. Danilin, D.D., Bivalves as potential indicators of areas of hydrothermal activity, in *Materialy konferentsii, posvyashchennoi Dnyu vulkanologa* (Proc. Conf. Dedicated to the Volcanologist's Day), Petropavlovsk-Kamchatsky: Inst. Vulkanol. Seismol., Dal'nevost. Otd. Ross. Akad. Nauk, 2013, pp. 291–294.
4. Drozdov, A.L. and Vinnikova, V.V., Morphology of gametes in sea urchins from Peter the Great Bay, Sea of Japan, *Russ. J. Dev. Biol.*, 2010, vol. 41, no. 1, pp. 37–45.
5. Drozdov, A.L. and Ivankov, V.N., *Morfologiya gamet zhivotnykh* (Morphology of Gametes in Animals), Moscow: Kruglyi God, 2000.
6. Drozdov, A.L. and Kasyanov, V.L., Size and shape of gametes in marine bivalve mollusks, *Biol. Morya*, 1985, no. 4, pp. 33–40.
7. Mashansky, V.F., Ozirskaya, E.V., Tumanova, N.L., and Drozdov, A.L., Intermitochondrial contacts in the telencephalon neurons of the lizard *Ophisaurus apodus, Tsitologiya*, 1984, vol. 26, no. 6, pp. 740–743.
8. Pashchenko, S.V. and Drozdov, A.L., Ultrastructure of gametes and acrosomal reaction of sperm in the bivalve *Glycymeris yessoensis, Tsitologiya*, 1991, vol. 33, no. 7, pp. 20–24.
9. Sagalevich, A.M., Torokhov, P.V., Matveenkov, V.V., et al., Hydrothermal manifestations at Piip's submarine volcano, Bering Sea, *Izv. Ross. Akad. Nauk, Ser. Geol.*, 1992, no. 9, pp. 104–114.
10. Audzijonyte, A., Krylova, E.M., Sahling, H., and Vrijenhoek, R.C., Molecular taxonomy reveals broad trans-oceanic distributions and high species diversity of deep-sea clams (Bivalvia: Vesicomyidae: Pliocardiinae) in chemosynthetic environments, *Syst. Biodiversity*, 2012, vol. 10, pp. 403–415.
11. Barry, J.P. and Kochevar, R.E., A tale of two clams: differing chemosynthetic life styles among vesicomyids in Monterey Bay cold seeps, *Cah. Biol. Mar.*, 1998, vol. 39, pp. 329–331.
12. Beninger, P. and Le Pennec, M., Reproductive characteristics of a primitive bivalve from a deep-sea reducing environment: giant gametes and their significance in *Acharax alinae* (Cryptodonta: Solemyidae), *Mar. Ecol.: Prog. Ser.*, 1997, vol. 157, pp. 195–206.

13. Berg, C.J., Reproductive strategies of mollusks from abyssal hydrothermal vent communities, *Bull. Biol. Soc. Wash.*, 1985, vol. 6, pp. 185–197.

14. Bieler, R., Mikkelsen, P.M., Collins, T.M., et al., Investigating the Bivalve Tree of Life – an exemplar-based approach combining molecular and novel morphological characters, *Invertebr. Syst.*, 2014, vol. 28, pp. 32–115.

15. Boss, K.J. and Turner, R.D., The giant white clam from the Galapagos Rift, *Calyptogena magnifica* species novum, *Malacologia*, 1980, vol. 20, pp. 161–194.

16. Coan, E.V., Scott, P.V., and Bernard, F.R., *Bivalve Seashells of Western North America: Marine Bivalve Mollusks from Arctic Alaska to Baja California*, Santa Barbara Museum of Natural History Monographs, vol. 2: Studies in Biodiversity, Santa Barbara, Calif.: Santa Barbara Mus. Nat. Hist., 2000.

17. Decker, C., Olu, K., Cunha, R.L., and Arnaud-Haond, S., Phylogeny and diversification patterns among vesicomyid bivalves, *PLoS One*, 2012, vol. 7, art. ID e33359.

18. Endow, K. and Ohta, S., Occurrence of bacteria in the primary oocytes of vesicomyid clam *Calyptogena soyoae, Mar. Ecol.: Prog. Ser.*, 1990, vol. 64, pp. 309–311.

19. Fiala-Medioni, A. and Le Pennec, M., Adaptive features of the bivalve molluscs associated with fluid venting in the subduction zones off Japan, *Palaeogeogr., Palaeoclimatol., Palaeoecol.*, 1989, vol. 71, pp. 161–167.

20. Franzén, Å., Comparative morphological investigations into the spermiogenesis among Mollusca, *Zool. Bidr. Uppsala*, 1955, vol. 30, pp. 399–456.

21. Franzén, Å., On spermiogenesis, morphology of the spermatozoon, and biology of fertilization among invertebrates, *Zool. Bidr. Uppsala*, 1956, vol. 31, pp. 355–482.

22. Franzé, Å., Ultrastructural studies of spermatozoa in three bivalve species with notes on evolution of elongated sperm nucleus in primitive spermatozoa, *Gamete Res.*, 1983, vol. 7, pp. 199–214.

23. Fujiwara, Y., Tsukahara, J., Hashimoto, J., and Fujikura, K., *In situ* spawning of a deep-sea vesicomyid clam: evidence for an environmental cue, *Deep Sea Res., Part I*, 1998, vol. 45, pp. 1881–1889.

24. Goffredi, S.K. and Barry, J.P., Species-specific variation in sulfide physiology between closely related Vesicomyid clams, *Mar. Ecol.: Prog. Ser.*, 2002, vol. 225, pp. 227–238.

25. Goffredi, S.K., Hurtado, L.A., Hallam, S., and Vrijenhoek, R.C., Evolutionary relationships of deep-sea vent and cold seep clams (Mollusca: Vesicomyidae) of the "*pacifica/lepta*" species complex, *Mar. Biol.*, 2003, vol. 142, pp. 311–320.

26. Healy, J.M., Molluscan sperm ultrastructure: correlation with taxonomic units within the Gastropoda, Cephalopoda and Bivalvia, in *Origin and Evolutionary Radiation of the Mollusca*, Oxford: Oxford Univ. Press, 1996, pp. 99–113.

27. Healy, J.M., Keys, J.L., and Daddow, L.Y.M., Comparative sperm ultrastructure in pteriomorphian bivalves with special reference to phylogenetic and taxonomic implications, *Geol. Soc. Spec. Publ.*, 2000, vol. 177, pp. 169–190.

28. Healy, J.M., Mikkelsen, P.M., and Bieler, R., Sperm ultrastructure in *Hemidonax pictus* (Hemidonacidae, Bivalvia, Mollusca): comparison with other heterodonts, especially Cardiidae, Donacidae and Crassateloidea, *Zool. J. Linn. Soc.*, 2008, vol. 153, pp. 325–347.

29. Heyl, T.P., Gilhooly, W.P., Chambers, R.M., et al., Characteristics of vesicomyid clams and their environment at the Blake Ridge cold seep, South Carolina, USA, *Mar. Ecol.: Prog. Ser.*, 2007, vol. 339, pp. 169–184.

30. Ikuta, T., Igawa, K., Tame, A., et al., Surfing the vegetal pole in a small population: extracellular vertical transmission of an 'intracellular' deep-sea clam symbiont, *R. Soc. Open Sci.*, 2016, vol. 3, art. ID 160130.

31. Johnson, S.B., Krylova, E.M., Audzijonyte, A., et al., Phylogeny and origins of chemosynthetic vesicomyid clams, *Syst. Biodiversity*, 2017, vol. 15, no. 4, pp. 346–360.

32. Kafanov, A.I. and Drozdov, A.L., Comparative sperm morphology and phylogenetic classification of recent Mytiloidea (Bivalvia), *Malacologia*, 1998, vol. 39, pp. 129–139.

33. Krylova, E.M. and Janssen, R., Vesicomyidae from Edison Seamount (South Western Pacific: Papua New Guinea: New Ireland fore-arc basin) (Bivalvia: Glossoidea), *Arch. Molluskenkd.*, 2006, vol. 135, pp. 233–263.

34. Krylova, E.M., Kolpakov, E.V., Sharina, S.N., et al., Distribution patterns of chemosymbiotic bivalves of the subfamily Pliocardiinae (Bivalvia: Vesicomyidae) from the North-West Pacific, in *15th Int. Deep-Sea Biol. Symp.*, September 9–14, 2018, Monterey, Calif.: Monterey Bay Aquarium Res. Inst., 2018, pp. 32–33.

35. Krylova, E.M. and Sahling, H., Recent bivalve molluscs of the genus *Calyptogena* (Vesicomyidae), *J. Molluscan Stud.*, 2006, vol. 72, pp. 359–395.

36. Krylova, E.M. and Sahling, H., Vesicomyidae (Bivalvia): current taxonomy and distribution, *PLoS One*, 2010, vol. 5, no. 4, art. ID e9957.

37. Krylova, E.M., Sahling, H., and Janssen, R., *Abyssogena*: a new genus of the family Vesicomyidae (Bivalvia) from deep-water vents and seeps, *J. Molluscan Stud.*, 2010, vol. 76, pp. 107–132.

38. Krylova, E.M., Sellanes, J., Valdés, F., and D'Elía, G., *Austrogena*: a new genus of chemosymbiotic bivalves (Bivalvia; Vesicomyidae; Pliocardiinae) from the oxygen minimum zone off central Chile described through morphological and molecular analyses, *Syst. Biodiversity*, 2014, vol. 12, pp. 225–246.

39. LaBella, A.L., Van Dover, C.L., Jollivet, D., and Cunningham, C.W., Gene flow between Atlantic and Pacific Ocean basins in three lineages of deep-sea clams (Bivalvia: Vesicomyidae: Pliocardiinae) and subsequent limited gene flow within the Atlantic, *Deep Sea Res., Part II*, 2016, vol. 137, pp. 307–317.

40. Le Pennec, M. and Beninger, P.G., Reproductive characteristics and strategies of reducing-system bivalves, *Comp. Biochem. Physiol., Part A: Mol. Integr. Physiol.*, 2000, vol. 126, pp. 1–16.

41. Lisin, E.S., Hannan, E.E., Kochevar, R.E., et al., Temporal variation in gametogenic cycles of vesicomyid clams, *Invertebr. Reprod. Dev.*, 1997, vol. 31, pp. 307–318.

42. Ockelmann, K.W., Developmental types in marine bivalves and their distribution along the Atlantic coast of Europe, in *Proc. 1st Eur. Malacol. Congr., London, 1962*, Cox, L.R. and Peake, J.F., Eds., London: Conchological Society of Great Britain and Ireland and the Malacological Society of London, 1965, pp. 25–35.

43. Parra, M., Sellanes, J., Dupré, E., and Krylova, E., Reproductive characteristics of *Calyptogena gallardoi* (Bivalvia: Vesicomyidae) from a methane seep area off Concepción, Chile, *J. Mar. Biol. Assoc. U. K.*, 2009, vol. 89, no. 1, pp. 161–169.

44. Peek, A.S., Gustafson, R.G., Lutz, R.A., and Vrijenhoek, R.C., Evolutionary relationships of deep-sea hydrothermal vent and cold-water seep clams (Bivalvia: Vesicomyidae): results from mitochondrial cytochrome oxidase subunit I, *Mar. Biol.*, 1997, vol. 130, pp. 151–161.

45. Popham, J.D., Comparative spermatozoon morphology and bivalve phylogeny, *Malacol. Rev.*, 1979, vol. 12, pp. 1–20.

46. Skulachev, V.P., Mitochondrial filaments and clusters as intracellular power transmitting cables, *Trends Biochem. Sci.*, 2001, vol. 26, no. 1, pp. 23–29.

Translated by T. Koznova